# Effects of S6-Strain *Mycoplasma gallisepticum* Inoculation at Ten, Twenty-Two, or Forty-Five Weeks of Age on the Egg Yolk Composition of Commercial Egg-Laying Hens[1,2]

E. D. Peebles,*[3] E. Y. Basenko,* S. L. Branton,† S. K. Whitmarsh,* D. V. Maurice,‡ and P. D. Gerard§

*Department of Poultry Science and §Experimental Statistics Unit, Mississippi State University, Mississippi State 39762; †Poultry Research Unit, Agricultural Research Service, United States Department of Agriculture, Mississippi State, 39762; and ‡Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC 340311

**ABSTRACT** Commercial laying hens maintained under controlled conditions were experimentally inoculated with the S6 strain of *Mycoplasma gallisepticum* (S6MG) at 45 wk of age. This resulted in depressed liver lipid concentration, and inoculations at 20 and 45 wk affected the size of various portions of the reproductive tract. In 2 consecutive trials of the current study, the effect of age of application of S6MG inoculation on the egg yolk characteristics of commercial layers similarly housed and maintained under controlled conditions was determined. The ages of inoculation compared were prior to lay at 10 wk of age, during onset of lay at 22 wk of age, and during postpeak lay at 45 wk of age. In each trial, yolk moisture and total lipid content were determined at 24, 32, 43, 47, and 58 wk of age. Yolk cholesterol concentration and yolk fatty acid profiles at wk 47 and 58 were also examined. Data from wk 24, 32, and 43 (effects of S6MG inoculations at 10 and 22 wk) and data from wk 47 and 58 (effects of S6MG inoculations at 10, 22, and 45 wk) were analyzed separately. The data of both trials were pooled then analyzed together. Across wk 47 and 58, percentage yolk lipid was significantly lower in eggs laid by birds inoculated at 10 wk compared with those inoculated at 45 wk. Sham-inoculated control and 22-wk inoculated groups had intermediate percentage yolk lipids. Compared with sham-control and 10-wk S6MG inoculation groups across wk 47 and 58, yolk myristic, oleic, and linolenic acid concentrations were reduced, whereas yolk stearic and arachidonic acid levels were increased by either 22- or 45-wk S6MG inoculations. In comparison with all other treatment groups at wk 47, yolk linoleic acid concentration was reduced by S6MG inoculation at 45 wk. Variable postpeak alterations in yolk total lipid and fatty acid content occur in response to the timing of S6MG inoculation in layers housed under controlled conditions.

**Key words**: fatty acid, layer, lipid, *Mycoplasma gallisepticum*, yolk

2006 Poultry Science 85:1502–1508

## INTRODUCTION

Mycoplasma gallisepticum (**MG**) infection causes egg production losses in adult laying flocks (Ley, 2003), and eggshell calcification may be affected by salpingitis in MG-infected birds (Domermuth et al., 1967). An F-strain MG (**FMG**) live vaccination at 12 wk subsequently alters liver, ovarian, and uterine characteristics (Burnham et al., 2002b) and yolk total lipid (**YTL**), cholesterol, and fatty acid concentrations (Burnham et al., 2003) in commercial layers. Specifically, concentrations of yolk linoleic, stearic, and arachidonic acids were increased, whereas concentrations of myristic, palmitoleic, and oleic acids were decreased by FMG treatment. It was suggested that FMG colonization in the liver of laying hens may affect egg production through alterations in YTL concentration and the metabolism and production of various fatty acids that are ultimately deposited in the yolk.

The various strains of MG may vary in virulence (Garcia et al., 1994), with the S6 strain of MG (**S6MG**) being considered one of the more virulent strains in the field (Levisohn et al., 1986). When S6MG was directly injected into the air sacs of specific-pathogen-free chickens, lesions were present in up to 90% of the experimental population (Lam et al., 1984). Deterioration in egg quality has been noted due to infection of the reproductive tract of chickens inoculated via the yolk sac with S6MG. In that study, 25% of the chickens had gross lesions of the oviduct, whereas 81% displayed microscopic lesions, found throughout all parts of the oviduct (Pruthi and Kharole, 1981). Nunoya et al. (1997) reported that S6MG infection


Received January 26, 2006.
Accepted March 22, 2006.
[1]This is journal article no. J-10826 from the Mississippi Agricultural and Forestry Experiment Station supported by MIS-321010.
[2]Use of trade names in this publication does not imply endorsement by the Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.
[3]Corresponding author: dpeebles@poultry.msstate.edu

caused salpingitis and up to a 4% decrease in monthly egg production in layers by 36 wk of age while housed in a commercial facility. More recent results showed that, under controlled conditions, S6MG inoculation at 10 wk of age produced no deleterious effect on the leukocytic characteristics (Peebles et al., 2004) or on egg production and internal egg and eggshell quality (Parker et al., 2002) of commercial layers. Conversely, a 20-wk S6MG inoculation significantly decreased the weights and lengths of various portions of the reproductive tracts of egg-laying hens (Parker et al., 2003). In companion articles, in which the same housing and population of birds were used as in the current study, S6MG inoculation at 45 wk significantly reduced eggshell quality (Basenko et al., 2005), and depressed liver lipid content and increased relative isthmus weight (Peebles et al., 2006).

Low-level MG infections can become exacerbated by the onset and increased rate of egg production (Yoder, 1991); therefore, it is important to follow birds over an entire laying cycle. The objective of the current study was to compare the effects of S6MG inoculation at 3 different ages throughout a complete egg-laying cycle on egg yolk composition in commercial layers. Comparison of the effects of S6MG challenges at various bird ages on egg yolk composition and their relationships to earlier reported performance traits and digestive and reproductive organ characteristics may provide further information as to the relative impact of S6MG inoculation and its age of application on layer metabolism.

## MATERIALS AND METHODS

### Pullet Housing and Management

Two individual trials were conducted using 1-d-old Single Comb White Leghorn pullets (Hy-Line W-36) obtained from a commercial source that was monitored and certified free of MG and *Mycoplasma synoviae* (**MS**; National Poultry Improvement Plan and Auxiliary Provisions, 2003). Chicks were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 12 d, chicks were vaccinated for Newcastle disease and infectious bronchitis by the same route then given a booster at 4 wk. At 9 wk of age in trial 1 and at 6 wk of age in trial 2, 10 randomly selected pullets were bled from the left vena cutanea ulnaris to obtain blood for the serum to be tested for antibodies to MG and MS using both the serum plate agglutination (**SPA**) and the hemagglutination-inhibition (**HI**) tests (Kleven, 1998). At those same times, swabs were also collected from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's-based (Papageorgiou medium) broth (Frey et al., 1968) supplemented with an additional 0.15 mg of thallium acetate and $10^6$ IU of penicillin G/mL. Tubes were incubated at 37°C for 30 d or until a phenol red indicator reaction occurred in the media indicating growth. Media samples from tubes that showed growth were inoculated onto Frey's-based agar and incubated at 37°C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody (**FA**) method (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-MG polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981). All initial mycoplasmal cultures as well as SPA and HI test results obtained from pullets at 9 wk of age in trial 1 and at 6 wk of age in trial 2 were negative for MG and MS.

Until the pullets were 10 wk of age, they were raised on clean, dry litter in a $5.5 \times 6.1$ m section of a conventional poultry house at the USDA-ARS Poultry Research Unit, resulting in an initial flock density of 0.034 $m^2$/bird. A daily artificial lighting schedule followed a cycle of 13 h of light per 11 h of dark. One 75-W incandescent light bulb was used to illuminate each 8.4 $m^2$ of floor space, providing a calculated intensity of 35.5 lx at bird level. At 10 wk of age, 11 pullets were randomly assigned to each of 16 (total of 176 pullets) negative pressure fiberglass biological isolation units (1.16 $m^2$). A controlled environment with limited exposure to bacteria and control of temperature, humidity, and lighting (Parker et al., 2002) was used. Isolation units with controlled environments were used to reduce or remove as many environmental stressors as possible so as to more accurately assess the true impact of the organism itself. The units were housed in a previously described poultry disease isolation facility at the same USDA research laboratory (Branton and Simmons, 1992). All birds were wing-banded for purposes of identification and individual data collection. The temperature inside each biological unit was maintained at 25°C.

### Layer Housing and Management

Bird numbers in each treatment replicate unit were reduced to 10 per unit (total of 160 pullets) at point-of-lay (18 wk of age) so that bird density was 0.116 $m^2$/bird for the duration of each trial. Of the 16 total units, 4 replicate units served as sham-inoculated controls throughout the study. Of the 12 other units, 4 replicate units were sequentially assigned to 1 of 3 inoculation treatment groups at the time of inoculation (treatment) so that by wk 45 (last treatment period) of each trial, 4 replicate units were assigned to each of 4 treatments with each treatment represented by 1 row of 4 units. More specifically, until the time at which birds in a set of 4 replicate units from a single row were treated (inoculated), they were included as control replicates. Four replicate units received S6MG inoculation at 10 wk of age (prior to lay), and 12 replicate units were designated as sham-inoculated controls from wk 10 through 21. Four of the 12 control units then received S6MG inoculation at 22 wk of age (onset of lay), and 8 replicate units then remained as controls from wk 22 through 44. Lastly, 4 of the 8 control units received S6MG at 45 wk of age (during lay), and 4 replicate units remained as controls from wk 45 through the end of each trial. In trial 2, the location of treatments within the isolation facility was different from that in trial 1 in an effort to remove potential treatment

positioning effects within the facility. Beginning at 18 wk of age, the duration of the artificial lighting schedule was increased 15 min/d until a cycle of 16 h 15 min of light per 7 h 45 min of dark was achieved in trial 1, and a cycle of 17 h 15 min of light per 6 h 45 min of dark was achieved in trial 2. These artificial lighting programs were maintained through the end of both trials.

## Pullet and Layer Diets

For the entirety of each trial, chickens had ad libitum access to feed and water. Diets in both trials were formulated according to the age of the birds and included the following: starter (0 to 6 wk), grower (7 to 12 wk), developer (13 to 18 wk), prelay (18 to 19 wk), and layer (20 to 60 wk). All diets were formulated to meet or exceed NRC (1994) specifications. Ingredient percentages and calculated analyses of these diets were as described by Burnham et al. (2002a). In both trials, CP and lysine percentages in the layer diet were adjusted according to the percentage of feed consumed per bird every 28 d until trial termination. No medication was administered during the study. Basenko et al. (2005) have reported the determined moisture, ash, CP, crude fat, crude fiber, total nitrogen, total digestible nitrogen, and fatty acid concentrations of feed and fecal samples collected at 54 wk of age in trial 2.

## S6MG Inoculation and Mycoplasma Detection

The S6MG-treated pullets were inoculated via eye drops in the right eye with 0.04 mL of a 24-h Frey's broth culture of high-passage S6MG at either 10, 22, or 45 wk of age. *Mycoplasma gallisepticum* organisms were passed in broth medium after being received from S. H. Kleven (University of Georgia, Athens) at the 212th passage. This S6MG strain was tested by a tracheal ring organ culture model as described by Cherry and Taylor-Robinson (1970) and was shown to significantly reduce ciliary activity within 5 d. Titers and passages of the experimental inocula in each trial were as previously described by Basenko et al. (2005). Similarly, pullets designated as controls were sham-inoculated via eye drops in the right eye at 10 wk of age with 0.04 mL of sterile Frey's broth medium. To ensure that all treatment groups were equally stressed by the inoculation process and to minimize the possibility of treatment group cross-contamination, control birds were sham-inoculated only once. At 60 wk of age in both trials, 1 randomly selected hen from each replicate unit in each of the 4 treatment groups was bled and swabbed to respectively test for antibodies to and culture for the organism. Each of these samples was tested for the presence of *Mycoplasma* species as previously described for pullets at 9 wk of age in trial 1 and at 6 wk of age in trial 2. Serum samples obtained from control birds at 60 wk of age in both trials were SPA and HI negative for MG, whereas the same tests were positive for MG in the S6MG-inoculated hens. Hens were considered MG-free when they exhibited no detectable HI titers.

## Data Collection

Eggs were collected for determination of yolk moisture and YTL concentrations at 24, 32, 43, 47, and 58 wk of age in both trials. Furthermore, yolk cholesterol concentration and yolk fatty acid profiles were examined at wk 47 and 58 in both trials. For determination of all of the above egg parameters, at least 10 eggs were collected from each replicate unit for an accurate estimate of each parameter (Buss, 1984). If fewer than 10 eggs were collected on a given day, more were collected the following day of the same week. Nevertheless, fresh yolk samples were taken on the same day that eggs were collected. Refrigerated samples collected on both days from the same replicate unit were pooled and were then weighed and processed for yolk moisture and YTL analysis. Individual samples were likewise pooled and frozen within each replicate prior to the analysis of all other specified parameters.

## Quantification of Yolk Moisture and Total Lipid Content

For analysis of yolk moisture content, duplicate fresh yolk samples (2 g) were dried according to the procedure of Peebles et al. (1999) in a commercial oven (model EL20, General Electric Co., Chicago Heights, IL). Yolk moisture contents were calculated as the difference between their wet and dry weights and were expressed as a percentage of wet yolk sample weight. For analysis of YTL content, lipid was extracted from duplicate fresh yolk samples (3 g) according to the procedure previously described by Bligh and Dryer (1959) and as modified by Latour et al. (1998). The YTL content was expressed as a percentage of fresh yolk sample weight. Yolk lipid samples were dissolved in 2 mL of hexane, 200 μL of 0.83% butylated hydroxytoluene, and refrigerated, as described by Christie (1982) for further content analyses as described below.

## Methyl Esterification of Yolk Lipids

Duplicate lipid samples were methylated according to the procedure described by Morrison and Smith (1964). A multiblock (Lab-Line Instruments Inc., Melrose Park, IL) system was used to boil each sample in a test tube at 80 ± 0.5°C for 30 min. A 200-μL aliquot of the solution was placed in a 2-mL gas chromatography vial, along with 400 μL of isooctane, and sealed with a rubber-lined cap for further fatty acid analyses by gas chromatography as described below.

## Chromatographic Analysis of Yolk Contents

Fatty acid profiles of duplicate yolk lipid samples were determined with a 5890 A Series I gas chromatograph (**GC**; Hewlett Packard Co., Boise, ID), according to the

procedure by Latour et al. (1998). Fatty acids were identified by comparing peak retention times against polyunsaturated fatty acids and rapeseed oil. The standards were injected periodically to ensure accurate measurement by the GC. The individual fatty acids retained by the GC were expressed as a percentage of the total fatty acid content of the fresh yolk sample. Yolk cholesterol concentration was determined by capillary gas-liquid chromatography after direct saponification and derivatization of fresh yolk samples (Maurice et al., 1994). Yolk cholesterol concentration was expressed in milligrams per gram of total yolk.

### Statistical Analysis

A completely randomized experimental design was utilized. Data from wk 24, 32, and 43 (after the 22 wk and prior to the 45-wk inoculations; first age interval) and from wk 47 and 58 (after the 45-wk inoculation; second age interval) were analyzed separately. It was necessary to analyze for the effects of treatment and bird age in each of the 2 age intervals separately, because the effects of the 45-wk S6MG inoculation could not be assessed in the first age interval, which ended prior to wk 45. The data of both trials were pooled then analyzed together. Trial was taken to be a random effect, and all data within each of the 2 age periods were subjected to a repeated-measures analysis to account for the fact that the same experimental units were observed over multiple age periods. In the first age interval, control, 10-wk S6MG-inoculated and 22-wk S6MG-inoculated groups were compared. In the second age interval, control, 10-wk S6MG-inoculated, 22-wk S6MG-inoculated, and 45-wk S6MG-inoculated groups were compared. Replicate means for each parameter were used in all data analyses. Results from trials 1 and 2 were not reported independently but were reported over both trials. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). All data were analyzed using the MIXED procedure of SAS Institute Inc. (2000), Version 8.1. Statements of significance were based on $P \leq 0.05$ unless otherwise stated.

## RESULTS

All S6MG-inoculated hens tested had HI titers ≥1:80 (geometric mean of 80.0). Similarly, FA culture results for swabs obtained at 60 wk of age in both trials were negative for *Mycoplasma* species growth for all control hens tested, whereas all S6MG-inoculated hens tested were MG positive (positive for MG fluorescence) and MS negative (negative for MS fluorescence).

Within the first (wk 24, 32, and 43 or after the 22 wk and prior to the 45-wk inoculation) and second (wk 47 and 58 or after the 45-wk inoculation) age intervals, there were no significant effects due to bird age or inoculation treatment on yolk moisture content. Further, there were no significant effects due to bird age or inoculation treatment on YTL concentration in the first age interval or on yolk cholesterol concentration in the second age interval. In the second age interval, there was also no significant bird age main effect or bird age by inoculation treatment interaction for YTL concentration. However, there was a significant ($P \leq 0.03$) main effect due to inoculation treatment on YTL concentration in the second age interval (Table 1). For the age interval encompassing wk 47 and 58, percentage YTL was significantly lower in eggs laid by birds inoculated at 10 wk compared with those inoculated at 45 wk, with sham-inoculated control and 22-wk inoculated groups intermediate.

In the second age interval (wk 47 and 58 or after the 45-wk inoculation), there were no significant effects due to bird age or inoculation treatment on yolk palmitic or palmitoleic acid concentrations. However, there were significant inoculation treatment main effects on yolk myristic ($P \leq 0.01$), stearic ($P \leq 0.004$), oleic ($P \leq 0.03$), linolenic ($P \leq 0.02$), and arachidonic ($P \leq 0.001$) acid concentrations in the second age interval (Table 1). There were no significant bird age main effects or bird age by inoculation treatment interactions for these fatty acids (myristic, stearic, oleic, linolenic, and arachidonic). Yolk myristic and oleic acid levels were significantly lower in eggs from 22- and 45-wk S6MG-inoculated hens compared with sham and 10-wk S6MG-inoculated hens. This relationship was also found for yolk linolenic acid, except that levels in eggs from 22-wk S6MG-inoculated birds were not significantly different from any of the other 3 treatment groups. Conversely, the opposite was true for yolk stearic and arachidonic acid concentrations in that stearic and arachidonic levels were higher in eggs from 22- and 45-wk S6MG-inoculated hens compared with sham and 10-wk S6MG-inoculated hens. There was a significant ($P \leq 0.003$) bird age by inoculation treatment interaction for yolk linoleic acid concentration (Table 2). Yolk linoleic acid concentration at wk 47 was significantly reduced by S6MG inoculation at 45 wk compared with all other treatment groups, whereas at wk 58, yolk linoleic acid concentration was not affected by inoculation treatment. For conciseness, only those data for which a significant inoculation treatment effect was noted are presented.

## DISCUSSION

At the end (60 wk of age in both trials) of this study, SPA, HI, and FA tests verified systemic infections in S6MG-inoculated birds. Conversely, sham-inoculated birds remained S6MG-free throughout the study. Manifestations of MG usually occur in the respiratory system, and lesions become extensive when complicated by other bacteria. Furthermore, environmental factors such as dust and ammonia, along with intensive rearing or stress, crowding, cold weather, live virus vaccination, or natural virus infection may also be important in lesion incidence and severity (Jordan, 1972; Springer et al., 1974; Jordan, 1985). However, when there are no secondary infections, mycoplasmosis is often subclinical or mild (Kerr and Olson, 1967). The birds in this study were housed in biological isolation units from 10 wk of age through the

**Table 1.** Yolk total lipid concentration and yolk myristic, stearic, oleic, linolenic, and arachidonic acid concentrations across 47 and 58 wk of age (second age interval) in sham-inoculated control (Control) and in 10- (S6MG-10), 22- (S6MG-22), and 45-(S6MG-45) wk S6MG inoculation treatments, trials 1 and 2[1]

| Treatment | Total lipid | Myristic acid | Stearic acid | Oleic acid | Linolenic acid | Arachidonic acid |
|---|---|---|---|---|---|---|
| | (%) | | | | | |
| Control | 29.8[ab] | 0.268[a] | 12.5[b] | 33.9[a] | 0.137[a] | 4.76[b] |
| S6MG-10 | 28.6[b] | 0.264[a] | 12.7[b] | 33.8[a] | 0.142[a] | 4.63[b] |
| S6MG-22 | 29.4[ab] | 0.240[b] | 13.7[a] | 32.6[b] | 0.116[ab] | 5.32[a] |
| S6MG-45 | 30.7[a] | 0.231[b] | 13.5[a] | 32.5[b] | 0.090[b] | 5.14[a] |
| SEM[2] | 1.58 | 0.0214 | 0.48 | 1.39 | 0.0177 | 0.280 |

[a,b]Means among treatments within column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 8 replicate units for the mean of each parameter within each treatment.

[2]SEM based on pooled estimate of variance.

remainder of the study, where they remained discernibly free of natural infections and other environmental stressors common in commercial operations. As a result, the S6MG-infected hens exhibited no outward pathological symptoms. Furthermore, as reported in a companion article by Basenko et al. (2005), in which the same birds were used as in this study, S6MG inoculation treatment had no significant effect on either mortality or BW.

In the companion report by Basenko et al. (2005), it was shown that 10-, 22-, or 45-wk S6MG inoculations also had no effect on egg production, egg weight, or relative yolk weight between 24 and 58 wk. Nevertheless, across 47 and 58 wk in the current study, YTL was significantly lower in eggs laid by hens inoculated with S6MG at 10 wk compared with eggs laid by hens inoculated at 45 wk. These results would, therefore, suggest that without affecting performance or egg and yolk weights, the timing of an S6MG inoculation may alter YTL. Upon comparing the fecal contents of sham, 10-, 22 -, and 45-wk S6MG-inoculated birds, Basenko et al. (2005) concluded that the 22-wk S6MG inoculation increased nutrient absorption over that in sham control birds. However, the lack of associated effects due to S6MG inoculation treatment on nutrient absorption and YTL would suggest that the depression in YTL after the 10-wk inoculation compared with the 45-wk inoculation was not due to a change in nutrient uptake in the gut per se. Alterations in liver lipid metabolism, the transport and delivery of lipids to the ovary, or the deposition of lipid in developing ovarian follicles remain as possible means by which differences in YTL may occur between 10- and 45-wk inoculation treatment groups. It was suggested by Burnham et al. (2002b), who used commercial layers housed in the same manner as those in the current study, that alterations in the performance and egg characteristics of layers involved mutual functional disturbances in the liver and ovary, without concomitant intestinal changes. This suggestion was supported by results showing a reduction in ripe ovarian follicle numbers and ovarian follicle size and increased incidences of fatty liver hemorrhagic syndrome in birds that demonstrated depressed performance subsequent to inoculation with FMG at 12 wk of age.

In another companion report (Peebles et al., 2006), it was noted that the same birds used in this study exhibited depressed liver lipid concentrations at 60 wk of age in response to the 45-wk S6MG inoculation. Although it was concluded in that report that the incidence of fatty liver hemorrhagic syndrome was not influenced by S6MG inoculation, the observed effect on liver lipid concentration would target the liver as a probable site through which subsequent yolk lipid content may be affected by an S6MG infection. Recent findings that show that MG has the ability to invade cells (Winner et al., 2000) suggest that MG may be capable of interfering with liver lipid metabolism. Furthermore, Winner et al. (2000) demonstrated that MG has the ability to pass through the mucosal barrier to cause systemic infections, and MG may be cultured from the avian liver (Sahu and Olson, 1976).

In addition to the effects of S6MG inoculation timing on postpeak (wk 47 and 58) YTL concentrations, postpeak yolk myristic, stearic, oleic, linoleic, linolenic, and arachidonic acid concentrations were also affected by S6MG inoculation and its timing. Overall, yolk myristic, oleic, and linolenic acid concentrations were reduced by the 22- and 45-wk inoculations compared with the sham and 10-wk inoculation groups, whereas the opposite relationship was noted for yolk stearic and arachidonic acid concentrations. The effects of a 12-wk FMG inoculation on yolk myristic, oleic, stearic, and arachidonic acids as reported by Burnham et al. (2003) reflected those due to the 22- and 45-wk S6MG inoculations in the current study. However, the effects of S6MG inoculation on yolk linoleic

**Table 2.** Yolk linoleic acid concentration in sham-inoculated control (Control) and in 10- (S6MG-10), 22- (S6MG-22), and 45- (S6MG-45) wk S6MG inoculation treatments at 47 and 58 wk of age (second age interval), trials 1 and 2[1,2]

| Treatment | Wk 47 | Wk 58 |
|---|---|---|
| | (%) | |
| Control | 13.8[a] | 13.1 |
| S6MG-10 | 13.5[a] | 13.2 |
| S6MG-22 | 13.7[a] | 13.2 |
| S6MG-45 | 12.7[b] | 13.4 |

[a,b]Means among treatments within column with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 4 replicate units for the mean of each parameter within each treatment.

[2]SEM based on pooled estimate of variance = 0.52.

acid were unlike those for myristic, oleic, and linolenic acids in that the effect was due only to the 45-wk inoculation, and it occurred only at wk 47. Nevertheless, these results would generally imply that the 22- and 45-wk S6MG inoculations caused postpeak elevations in yolk stearic and arachidonic acid concentrations at the expense of myristic, oleic, linoleic, and linolenic acid concentrations. More specifically, S6MG infection at 22 and 45 wk may affect fatty acid elongation and desaturation processes in the liver endoplasmic reticula of these birds. Typical hepatic desaturation in the de novo synthesis of oleic acid from stearic acid (Klasing, 1998) may be inhibited by the 22- and 45-wk S6MG inoculations. It is further suggested that liver microsomal cytochrome $b_5$-oxygenase activity in the sequential desaturations of linoleic acid and linolenic acid for the subsequent formation of arachidonic acid (Lehninger, 1975; Klasing, 1998) may be stimulated by S6MG infections during lay. The fatty acid synthetase system, which is responsible for the elongation of short-chain fatty acids, including myristic acid, to form stearic acid, and the elongation of linolenic acid, to form arachidonic acid (Lehninger, 1975; Klasing, 1998), may likewise be promoted by the colonization of S6MG in the liver during lay.

Basenko et al. (2005) suggested that layers may be better able to adapt to an S6MG infection when inoculated prior to or at the onset of lay rather than late in lay. The decrease in liver lipid content in response to the 45-wk inoculation compared with the absence of an effect after the 10- or 22-wk inoculations reported by Peebles et al. (2006) would further indicate that livers are more susceptible to the effects of S6MG infection at 45 wk than at prelay or at the initiation of lay in commercial laying hens. An active S6MG infection may, therefore, cause livers in birds to react earlier in the reproductive cycle in ways similar to that of inefficient or aging hens. However, despite the possible S6MG inoculation time effects on the liver, the prelay inoculation depressed YTL compared with the inoculation given late in lay, and both early and late inoculations during lay changed the concentrations of various yolk fatty acids in relation to concentrations after the prelay inoculation. Therefore, the effects of S6MG inoculation timing on liver lipid concentration do not directly correspond to those effects on yolk lipid content. Nevertheless, it may be concluded that variable postpeak alterations in yolk lipid and fatty acid content may occur in response to the timing of S6MG inoculation in layers housed under controlled conditions. Furthermore, because these birds were housed in biological isolation units, these results do not preclude the possibility that different or greater effects on egg yolk composition may occur in birds infected prelay, at lay onset, or late in lay with S6MG when housed in facilities where there are increased levels of environmental stress.

## ACKNOWLEDGMENTS

This work was funded by grant no. 58-6406-9-016 from the United States Department of Agriculture.

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